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Editorial

Monitoring antiviral resistance in patients receiving nucleos(t)ide analog therapies for hepatitis B: Which method should be used? ☆

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Antiviral drug resistance is a major hurdle to the success of nucleos(t)ide analog therapy for hepatitis B. Among nucleoside-naïve patients, antiviral drug resistance had been reported in up to 70% of patients after 4 years of lamivudine (LVD), 29% after 5 years of adefovir, 1% after 4 years of entecavir (ETV), and 9–22% after 2 years of telbivudine [1–4]. Resistance rates are substantially higher in patients with prior resistance to LVD, with rates of up to 20% after 2 years of adefovir and 40% after 4 years of entecavir [3,5]. Sequential therapy had also been reported to result in the selection of multi-drug resistant HBV [6,7].

A fundamental issue regarding antiviral resistance is the criteria for defining drug-resistant mutations and the method used to diagnose drug resistance [8]. In clinical practice, virologic breakthrough (defined as a greater than 1 log increase in serum hepatitis B virus [HBV] DNA from nadir during treatment) is often the only method used to diagnose drug resistance. However, breakthroughs are not always due to drug resistance. Clinical trials found that 30–50% of patients with virologic breakthroughs had no detectable antiviral drug-resistant mutations indicating that non-adherence may be the reason for the breakthroughs [9–13]. Empiric modification of treatment based on presumed diagnosis of drug resistance may unnecessarily expose patients to sequential therapies and eventually exhaust all available treatments.

On the other hand, failure to detect antiviral drug-resistant mutations does not exclude the possibility that breakthrough was related to drug resistance. Drug-resistant mutations may be present but were not detected due to lack of sensitivity of the method used. Direct (or population) sequencing of PCR-amplified HBV DNA is the most common method used for detecting antiviral drug-resistant mutations but these mutations need to reach approximately 20% of the total HBV quasispecies pool to be detected. Restriction fragment length polymorphism (RFLP) analyses and reverse hybridization assay (line probe, LiPA DR) are more sensitive and can detect drug-resistant mutations that comprise 5% of the total viral population [14–17]. However, these assays can only detect a limited number of previously characterized mutations. Furthermore, RFLP analysis may not be possible for all resistant mutations as endonucleases specific for such sequences may not exist. Mutations may be missed because they are not captured by the assay e.g. mutations located outside the reverse transcriptase (RT) domain of the HBV polymerase gene and mutations not detected by any of the probes in the line probe assay. In this regard, sequencing using microchip-based technology using oligonucleotide microarrays has the advantage that the entire viral genome can be scanned

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in one assay [18]. Furthermore, mutations may not be recognized if they have not been reported previously; this would particularly be difficult if baseline HBV sequences were not available for comparison. As an increasing number of patients with divergent HBV sequences are exposed to nucleos(t)ide analog treatments, additional mutations in the reverse transcriptase domain other than the signature resistance mutations may be identified, e.g. alanine to threonine substitution at position 181 (rtA181T) [19] and methionine to serine substitution at position 204 (rtM204S) [20] had been reported to be associated with LVD resistance in addition to methionine to valine or isoleucine substitution at position 204 (rtM204V/I).

Caution must be exercised in attributing changes in the HBV RT domain as the cause of antiviral drug resistance. The significance of several mutations such as rtA181T and secondary resistance to adefovir [21,22], rtI233V and primary resistance to adefovir [23,24] and rtA194T and secondary resistance to tenofovir [25,26] remain controversial. Thus, phenotypic assays are necessary to confirm that a newly identified mutation(s) is associated with drug resistance and to define the spectrum of mutations associated with resistance to new therapies.

In this issue of the Journal, Baldick et al. present a comprehensive phenotypic analysis of 511 patient isolates collected from clinical trials of ETV therapy in nucleoside-naïve and in LVD-refractory hepatitis B patients [27]. In previous studies, the authors demonstrated that mutations associated with resistance to LVD decrease susceptibility to ETV by 8-fold and addition of mutations at rtT184, S202, and M250 further decrease susceptibility to ETV by approximately 10-fold [28]. In the current study, the authors first investigated the correlation between genotypic resistance and ETV susceptibility using laboratory clones. Then, they repeated the same studies using patient-derived clones. The authors found that laboratory clones with rtM204V and rtL180M substitutions displayed 8-fold reduced ETV susceptibility, and addition of rtT184L, rtS202G or rtM250V substitutions further reduces ETV susceptibility by approximately 100-fold relative to wild-type HBV. They also showed that patient isolates displayed a similar relationship between genotypic and phenotypic resistance as compared to laboratory clones although the variability in ETV susceptibility was greater with patient derived clones. These findings are important as phenotypic assays are time-consuming [8,29]. That the same results can be obtained by introducing a variety of mutations to laboratory clones versus creating separate clones derived from individual patient isolates enable phenotypic confirmation of newly identified putative drug-resistant mutations to be performed more expeditiously. The authors noted that while the results of individual patient clones generally correlated with 'population phenotyping' in which the entire patient isolate quasispecies is cloned en masse to mimic the circulating virus pool, a decrease in ETV susceptibility was observed only when clones with ETV-resistant mutations approach 25% of the virus population.

Previous reports from these authors [28,30] and some sections of the current paper indicated that the backbone for ETV resistance is a combination of rtL180M and rtM204V. However, data in this current study clearly showed that rtM204I alone has the same effect (Figs. 1B and 2, and Table 2). Clarification of this point is important as patients with telbivudine resistance which is associated with rtM204I but not rtM204V [31] would also have diminished response to ETV and increased risk of ETV resistance.

The authors observed a range in ETV resistance in the phenotypic studies with rtS202G/C substitutions, some rtT184 substitutions, and multiple ETV resistance substitutions being associated with the highest level of resistance. These data need to be verified as only 1 or 2 clones from patient isolates were tested for some substitutions. Until further data are available, all the reported substitutions including those involving rtM250 should be considered to be associated with ETV resistance.

An important contribution of Baldick et al.'s study is the careful analysis of isolates from patients with virologic breakthrough but no detectable substitutions at rt184, 202 and 250. The authors performed phenotypic studies using full-length patient isolates and recombinants created with the patients' RT domain obtained at the time of virologic breakthrough and found similar ETV susceptibility as wild-type HBV clones as well as isolates from these patients obtained at baseline. By contrast, studies using isolates from patients with LVD or ETV resistance substitutions showed the expected ETV susceptibility profiles whether patient RT or full-length virus sequence was used. These data indicate that virologic breakthrough in patients with no detectable substitutions at rt184, 202 and 250 is likely related to non-compliance with medications and not to unidentified mutations in the RT domain or elsewhere in the HBV genome.

How would the results of this study influence antiviral resistance monitoring of hepatitis B patients receiving nucleos(t)ide analog therapies? Serum HBV DNA monitoring to detect virologic breakthrough remains the most practical method in clinical practice. Testing for genotypic resistance should be performed in patients with virologic breakthrough. Treatment should be modified in patients found to have signature resistance mutations. Medication compliance should be reinforced in patients with no detectable resistance mutations. Additional testing using more sensitive methods for genotypic resistance may be performed. Alternatively, genotypic resistance can be repeated in patients with persistent virologic breakthrough despite medication

compliance. Substitutions in the HBV RT domain that had not been reported to be associated with resistance to the antiviral drug administered should be tested in phenotypic assays. Laboratory strains with the identified substitutions introduced by site directed mutagenesis would suffice in most instances but confirmation of phenotypic resistance using selected patient isolates should still be performed when evaluating resistance to new antiviral drugs. Only substitutions shown to reduce susceptibility in carefully conducted phenotypic studies should be reported as resistance-associated mutations. These recommendations are in line with those of an international panel [8].

References

[1] Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology 2003;125:1714–1722.

[2] Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006;131:1743–1751.

[3] Colonno RT, Rose RE, Pokornowski K, Baldick CT, Eggers B. Four year assessment of ETV resistance in nucleoside-naive and lamivudine refractory patients. J Hepatol 2007;46:S294.

[4] Lai C, Gane E, Hsu CW, Thongsawat S, Wang Y. Two-year results from the globe trial in patients with hepatitis B: greater clinical and antiviral efficacy for telbivudine (LdT) vs. lamivudine. Hepatology 2007;46:63A.

[5] Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006;44:283–290.

[6] Villet S, Pichoud C, Villeneuve JP, Trepo C, Zoulim F. Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient. Gastroenterology 2006;131:1253–1261.

[7] Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 2006;44:703–712.

[8] Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007;46:254–265.

[9] Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia hepatitis lamivudine study group. N Engl J Med 1998;339:61–68.

[10] Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256–1263.

[11] Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001–1010.

[12] Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576–2588.

[13] Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011–1020.

[14] Kim HS, Han KH, Ahn SH, Kim EO, Chang HY, Moon MS, et al. Evaluation of methods for monitoring drug resistance in chronic hepatitis B patients during lamivudine therapy based on mass spectrometry and reverse hybridization. Antivir Ther 2005;10:441–449.

[15] Lok AS, Zoulim F, Locarnini S, Mangia A, Niro G, Decraemer H, et al. Monitoring drug resistance in chronic hepatitis B virus (HBV)-infected patients during lamivudine therapy: evaluation of performance of INNO-LiPA HBV DR assay. J Clin Microbiol 2002;40:3729–3734.

[16] Hussain M, Fung S, Libbrecht E, Sablon E, Cursaro C, Andreone P, et al. Sensitive line probe assay that simultaneously detects mutations conveying resistance to lamivudine and adefovir. J Clin Microbiol 2006;44:1094–1097.

[17] Allen MI, Gauthier J, DesLauriers M, Bourne EJ, Carrick KM, Baldanti F, et al. Two sensitive PCR-based methods for detection of hepatitis B virus variants associated with reduced susceptibility to lamivudine. J Clin Microbiol 1999;37:3338–3347.

[18] Tran N, Berne R, Chann R, Gauthier M, Martin D, Armand MA, et al. European multicenter evaluation of high-density DNA probe arrays for detection of hepatitis B virus resistance mutations and identification of genotypes. J Clin Microbiol 2006;44:2792–2800.

[19] Yeh CT, Chien RN, Chu CM, Liaw YF. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. Hepatology 2000;31:1318–1326.

[20] Bozdayi AM, Uzunalimoglu O, Turkyilmaz AR, Aslan N, Sezgin O, Sahin T, et al. YSDD: a novel mutation in HBV DNA polymerase confers clinical resistance to lamivudine. J Viral Hepat 2003;10:256–265.

[21] Borroto-Esoda K, Miller MD, Arterburn S. Pooled analysis of amino acid changes in the HBV polymerase in patients from four major adefovir dipivoxil clinical trials. J Hepatol 2007;47:492–498.

[22] Lacombe K, Ollivet A, Gozlan J, Durantel S, Tran N, Girard PM, et al. A novel hepatitis B virus mutation with resistance to adefovir but not to tenofovir in an HIV-hepatitis B virus-co-infected patient. Aids 2006;20:2229–2231.

[23] Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, et al. Variant of hepatitis B virus with primary resistance to adefovir. N Engl J Med 2006;354:1807–1812.

[24] Curtis M, Zhu Y, Borroto-Esoda K. Hepatitis B virus containing the I233V mutation in the polymerase reverse-transcriptase domain remains sensitive to inhibition by adefovir. J Infect Dis 2007;196:1483–1486.

[25] Sheldon J, Camino N, Rodes B, Bartholomeusz A, Kuiper M, Tacke F, et al. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. Antivir Ther 2005;10:727–734.

[26] Delaney 4th WE, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, et al. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob Agents Chemother 2006;50:2471–2477.

[27] Baldick CJ, Eggers BJ, Fang J, Levine SM, Pokornowski KA, Rose RE, et al. Hepatitis B virus quasispecies susceptibility to entecavir confirms the relationship between genotypic resistance and patient virologic response. J Hepatol 2008;48:895–902.

[28] Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. Antimicrob Agents Chemother 2004;48:3498–3507.

[29] Zoulim F. In vitro models for studying hepatitis B virus drug resistance. Semin Liver Dis 2006;26:171–180.

[30] Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology 2006;44:1656–1665.

[31] Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, et al. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528–536.